

AMENDMENTS TO THE CLAIMS

The following list of claims will replace all prior versions and lists of claims in the application.

Listing of Claims:

1. (Currently amended) A method for ~~separating~~ enriching an isotopes of an actinide element comprising steps of:
 - (a) ~~providing a composition comprising molecules comprising an actinide element, wherein at least some of the molecules include a first isotope and of the actinide element and at least some of the molecules include a second isotope of the actinide element; and~~
 - (b) ~~exposing the molecules comprising the actinide element contacting the composition with microorganisms that have to~~ reducing activity of the actinide element ~~reducing microorganisms, wherein the microorganisms preferentially reduce the second isotope of the actinide element and~~ thereby allowing formation of a ~~precipitate comprising the actinide element, wherein the precipitate second composition comprising reduced actinide element~~ containing a higher proportion of the second isotope relative to the first isotope than was present in the original composition, thereby ~~effecting enriching the second isotope of the actinide element, a separation of the first and second isotopes; and~~
~~effecting an increased separation of the first and second isotopes present in the precipitate using any suitable process.~~
2. (Currently amended) The method of claim 1, wherein ~~the exposing step comprises:~~
 - ~~combining the composition and the microorganisms in a vessel together with is provided in a culture medium, thereby producing a culture; and~~
 - ~~maintaining the culture for a time sufficient to allow formation of a precipitate.~~
3. (Currently amended) The method of claim 1, wherein the second composition is insoluble, further comprising the step of:

— ~~separating the precipitate from unprecipitated molecules containing the actinide element.~~

4. (Currently amended) The method of claim 3, wherein ~~the method further comprises a~~ the step of separating comprises collecting the second composition~~precipitate.~~
5. (Currently amended) The method of claim 3, wherein ~~the method further comprises a~~ the step of separating comprises collecting the second composition~~precipitate~~ and the microorganisms.
6. (Currently amended) The method of claim 5, ~~wherein the method further comprising the a step of removing from treating the microorganisms to remove molecules the adsorbed composition containing adsorbed, unreduced actinide element atoms therefrom.~~
7. (Currently amended) The method of claim 1, wherein ~~the exposing step (b) is performed for a time period between 0.05 and 100 hours~~selected to achieve optimum isotope separation.
8. (Canceled)
9. (Currently amended) The method of claim ~~7~~4, wherein ~~the exposing step (b) is performed for a time period between 0.1 and 50 hours~~selected to achieve reduction of less than 80% of the actinide element.
10. (Currently amended) The method of claim ~~7~~4, wherein ~~the exposing step (b) is performed for a time period between 0.1 and 20 hours~~selected to achieve reduction of less than 60% of the actinide element.
11. (Currently amended) The method of claim ~~7~~4, wherein ~~the exposing step (b) is performed for a time period between 0.1 and 10 hours~~selected to achieve reduction of less than 40% of the actinide element.

12. (Currently amended) The method of claim 74, wherein the exposing step (b) is performed for a time period between 0.1 and 5 hours~~selected to achieve reduction of less than 20% of the actinide element.~~
- 13-21. (Canceled)
22. (Currently amended) The method of claim 1, wherein the method further comprising the a step of:
determining the isotope content of the second composition~~precipitate.~~
23. (Currently amended) The method of claim 1, wherein the method effecting step further comprises steps of:
(c) ~~converting~~ re-oxidizing the reduced actinide element present in the second composition ~~into a form suitable for repetition of the exposing step; and~~
(d) repeating steps (a) and (b) ~~the exposing step~~ using the re-oxidized converted actinide element as a starting material, thereby further enriching the second isotope of the actinide element~~effecting an increased separation of the first and second isotopes.~~
- 24-36. (Canceled)
37. (Original) The method of claim 1, wherein the steps are performed in batch mode.
38. (Original) The method of claim 1, wherein the steps are performed in continuous mode.
39. (Canceled)
40. (Currently amended) The method of claim 1, wherein the microorganisms are ~~present in a medium that is separated from the composition~~ by a semi-permeable membrane ~~during the exposing step, which wherein the~~ semi-permeable membrane allows diffusion of the composition comprising the first and second isotope of the actinide element containing molecules but does not permit passage of the microorganisms.

41. (Currently amended) The method of claim 40, wherein ~~the exposing step (b)~~ comprises allowing the composition to flow continuously past the semi-permeable membrane, thereby ~~allowing diffusion of the actinide element containing molecules so as to expose them to the reducing activity of~~ contacting the microorganisms.
42. (Canceled)
43. (Currently amended) The method of claim ~~40~~42, wherein the microorganisms are present in a medium and wherein the composition and the medium ~~containing the microorganisms~~ flow in opposite directions.
44. (Currently amended) The method of claim 1, further comprising ~~the a~~ step of: immobilizing the microorganisms prior to performing ~~the exposing step (b)~~.
45. (Original) The method of claim 44, wherein the step of immobilizing the microorganisms comprises:
fixing the microorganisms to a solid support.
46. (Original) The method of claim 44, wherein the microorganisms are contained within a semi-permeable membrane or membranes.
47. (Currently amended) The method of claim 44, wherein ~~the exposing step (b)~~ comprises allowing the composition to traverse the immobilized microorganisms.
48. (Original) The method of claim 1, wherein the actinide element is uranium.
49. (Currently amended) The method of claim 48, wherein ~~the precipitate~~ second composition comprises uraninite.
50. (Original) The method of claim 48, wherein the microorganisms reduce uranium from the U(VI) state to the U(IV) state.

51. (Original) The method of claim 48, wherein the first isotope is heavier than the second isotope.
52. (Original) The method of claim 48, wherein the first uranium isotope is U-238.
53. (Original) The method of claim 48, wherein the second uranium isotope is U-235.
54. (Original) The method of claim 48, wherein the first uranium isotope is U-238 and the second uranium isotope is U-235.
55. (Original) The method of claim 1, wherein the method achieves a separation factor of at least 1.02.
56. (Original) The method of claim 1, wherein the method achieves a separation factor of at least 1.06.
57. (Original) The method of claim 1, wherein the method achieves a separation factor of at least 1.10.
58. (Withdrawn) The method of claim 1, wherein the actinide element is plutonium.
59. (Withdrawn) The method of claim 1, wherein the actinide element is neptunium.
60. (Original) The method of claim 1, wherein the composition comprises an electron donor.
61. (Currently amended) The method of claim 1, wherein the ~~molecules~~ composition ~~comprising the actinide element are provided together with a counter ions,~~ wherein the counter ions ~~is~~ are not an electron accepting species that competes with the actinide element for reduction by the microorganisms.
62. (Withdrawn) The method of claim 61, wherein the counter ion can serve as a substrate for metabolism by the microorganisms under anaerobic conditions.

63. (Currently amended) The method of claim 61, wherein the counter ions ~~is~~ are selected from the group consisting of: lactate, acetate, and chloride.
64. (Original) The method of claim 1, wherein the microorganisms are metal or sulfate reducing bacteria.
65. (Original) The method of claim 1, wherein the microorganisms are facultative aerobes.
66. (Withdrawn) The method of claim 1, wherein the microorganisms are anaerobes.
67. (Original) The method of claim 1, wherein the microorganisms are members of a bacterial genus selected from the group consisting of: *Clostridium*, *Shewanella*, *Geobacter*, *Pyrobaculum*, *Desulfotomaculum*, and *Desulfovibrio*.
68. (Original) The method of claim 67, wherein the microorganisms are members of bacterial genus *Shewanella*.
69. (Original) The method of claim 67, wherein the microorganisms are members of bacterial strain *Shewanella oneidensis*.
70. (Withdrawn) The method of claim 67, wherein the microorganisms are selected from the group consisting of *Clostridium* sp., *Deinococcus radiodurans* R1, *Geobacter chapelleii*, *Geobacter hydrogenophilus* H2, *Geobacter hydrogenophilus* H2, *Geobacter* H4, *Geobacter* TACP-2, *Geobacter* TACP-3, *Pyrobaculum islandicum*, *Shewanella alga*, *Shewanella saccharophila*, *Desulfotomaculum reducens* MI-1, *Desulfovibrio desulfuricans*, and *Desulfovibrio vulgaris*.
71. (Currently amended) The method of claim 1, wherein the microorganisms are present at a concentration of between approximately 10^7 and 10^9 per milliliter ~~during the exposing step~~.
72. (Original) The method of claim 1, wherein the microorganisms overexpress a gene encoding a protein that reduces the actinide element.

73. (Original) The method of claim 72, wherein the gene encodes a cytochrome c protein.
74. (Original) The method of claim 73, wherein the cytochrome c is a cytochrome c3.
75. (Original) The method of claim 1, wherein the microorganisms overexpress a gene encoding a protein involved in a pathway leading to reduction of the actinide element.
76. (Original) The method of claim 1, wherein the microorganisms express an altered cytochrome c protein, which altered cytochrome c protein displays an increased ability to reduce the actinide element relative to a wild type version of the protein.
77. (Canceled)
78. (Currently amended) The method of claim 1, wherein ~~the exposing step (b)~~ takes place in a medium substantially free of counter ions capable of forming insoluble salts with unreduced ~~molecules of the~~ actinide element.
- 79-81. (Canceled)
82. (Currently amended) The method of claim 78, wherein the medium ~~lacks significant amounts~~ is substantially free of phosphate.
83. (Original) The method of claim 1, wherein the exposing step (b) is performed in the presence of an organic polymer.
84. (Currently amended) The method of claim 1, wherein the method further comprising the a step of:
culturing the microorganisms under aerobic conditions prior to ~~the maintaining~~
step (b) and performing ~~the maintaining step (b)~~ under anaerobic conditions.
- 85-88. (Canceled)

89. (Currently amended) The method of claim ~~186~~, wherein the microorganisms are thermophilic bacteria, and wherein ~~the exposing step (b)~~ is performed at a temperature above 50°C.
90. (Currently amended) The method of claim ~~186~~, wherein the second composition is soluble~~further comprising the step of:~~
~~—extracting some or all of the reduced molecules from the composition.~~
91. (Currently amended) The method of claim 90, wherein the method further comprises ~~ing~~ the a step of:
~~reextracting the second composition comprising the reduced actinide element containing molecules into an separate aqueous phase and either reoxidizing them or collecting them.~~
92. (Currently amended) The method of claim ~~91~~~~90~~, wherein the method further comprises ~~the extracting a step of~~comprises:
 forming an extractable organic complex comprising reduced actinide ~~molecules~~ element using an organic ligand; ~~and~~
~~—extracting the extractable complex into an organic phase or onto a solid support coated with an organic material.~~
93. (Original) The method of claim 92, wherein the organic ligand comprises thenoyltrifluoroacetone.
- 94-100. (Canceled)
101. (Currently amended) A method for ~~separating~~enriching an isotopes of an actinide element comprising steps of:
 (a) providing a composition comprising ~~molecules comprising an actinide element, wherein at least some of the molecules include a first isotope of the actinide element and at least some of the molecules include and~~ a second isotope of the actinide element; and

(b) incubating the composition ~~molecules comprising the actinide element~~ with an actinide reducing enzyme obtained from ~~an actinide element-reducing microorganism that~~ has reducing activity of the actinide element, thereby ~~allowing formation of a precipitate comprising the actinide element, wherein the precipitate contains forming a second composition comprising the reduced actinide element containing a higher proportion of the second isotope relative to the first isotope than was present in the original composition, thereby enriching the second isotope of the actinide element, effecting a separation of the first and second isotopes; and~~ effecting an increased separation of the first and second isotopes present in the precipitate using any suitable process.

102. (Currently amended) The method of claim 101, wherein the second composition is insoluble, further comprising the step of:
—— ~~separating the precipitate from unprecipitated molecules containing the actinide element.~~
103. (Currently amended) The method of claim 102, wherein the method further comprises a the step of separating comprises collecting the second composition using an organic polymer, precipitate.
- 104-120. (Canceled)
121. (Original) The method of claim 101, wherein the steps are performed in batch mode.
122. (Original) The method of claim 101, wherein the steps are performed in continuous mode.
123. (Original) The method of claim 101, wherein the enzyme is at least partially purified.
124. (Original) The method of claim 101, wherein an electron donor is present during the incubating step.

125. (Original) The method of claim 101, wherein the actinide reducing enzyme is a cytochrome c enzyme.
126. (Original) The method of claim 125, wherein the cytochrome c is a cytochrome c3.
127. (Original) The method of claim 125, wherein the cytochrome c is at least partially purified.
128. (Currently amended) The method of claim 101, wherein a hydrogenase obtained from an actinide element reducing the microorganism is present during the incubating step.
129. (Original) The method of claim 128, wherein the hydrogenase is at least partially purified.
130. (Original) The method of claim 128, wherein an electron donor in addition to hydrogenase is present during the incubating step.
131. (Canceled)
132. (Currently amended) The method of claim 101, wherein a material providing a nucleation site to precipitate the second composition for uraninite formation is provided during the incubating step.
133. (Original) The method of claim 101, wherein the incubating step is performed in a fixed enzyme reactor.
- 134-154. (Canceled)
155. (New) The method of claim 101, wherein the actinide element is uranium.
156. (New) The method of claim 155, wherein the second composition comprises uraninite.
157. (New) The method of claim 155, wherein the enzyme reduces uranium from the U(VI) state to the U(IV) state.

158. (New) The method of claim 155, wherein the first isotope is heavier than the second isotope.
159. (New) The method of claim 155, wherein the first uranium isotope is U-238.
160. (New) The method of claim 155, wherein the second uranium isotope is U-235.
161. (New) The method of claim 155, wherein the first uranium isotope is U-238 and the second uranium isotope is U-235.
162. (New) The method of claim 1, wherein the actinide element is uranium and the microorganisms are members of bacterial genus *Shewanella*.
163. (New) The method of claim 162, wherein the actinide element is uranium and the microorganisms are members of bacterial strain *Shewanella oneidensis*.
164. (New) The method of claim 162, wherein the first uranium isotope is U-238 and the second uranium isotope is U-235.